CheKine™ Triglyceride (TG) Colorimetric Assay Kit

Item NO.	Product Name
KTB2200	CheKine™ Triglyceride (TG) Colorimetric Assay Kit

ATTENTION

For laboratory research use only. Not for clinical or diagnostic use

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INTRODUCTION

Background

Triglycerides (TG) are fat molecules formed by long-chain fatty acids and glycerol. They are not only the main components of cell membranes, but also important respiratory substrates. Serum triglycerides (TG) is an important index for clinical blood lipid measurement.

Assay Principle

The kit provides a simple method for detecting TG concentration in a variety of biological samples such as serum, plasma, tissues, cells, and plants. In the assay, TG can be extracted by isopropanol and then saponified by KOH to produce glycerol and fatty acid. Further, periodic acid oxidizes glycerin to form formaldehyde. In the presence of chloride ions, formaldehyde can react with acetylacetone to form a yellow substance which has a characteristic absorption peak at 420 nm. The triglyceride (TG) present in the sample is proportional to the signal obtained.

Storage/Stability

Storage at 4°C and protected from light upon receipt. Kit has a storage time of 12 months form receipt. Refer to list of materials supplied section for storage conditions of individual components.

Technical hints

- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if used separately or substituted.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross-contamination, change pipette tips between additions of standards, samples and reagents. Also, use separate reservoirs for each reagent.
- Ensure all reagents and equipment are at the appropriate temperature before starting the assay.
- There are volatile substances in the kit, gloves and masks should be worn during the experiment, and the reagent bottle cap should be closed in time after opening.
- After adding Reagent I, shake vigorously to fully extract triglycerides in serum
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.

PRODUCT INFORMATION

Materials Supplied and Storage Conditions

Kit components	Quantity		Storage conditions
	48 T	96 T	
Reagent I	2 mL	4 mL	4°C
Reagent II	1 mL	2 mL	4°C protected from light
Reagent III	5 mL	10 mL	4°C protected from light
Reagent IV	5 mL	10 mL	4°C protected from light
Standard	1 mL	1 mL	4°C protected from light

Other Materials Required, Not Supplied

- Standard microplate reader capable of measuring absorbance at OD420 nm
- Precision pipettes, disposable pipette tips
- Distilled or deionized water
- 96 well plate with clear flat bottom
- Centrifuge, Incubator, Ice Maker
- Redistilled water
- Dounce homogenizer (for tissue samples).
- Isopropanol.

ASSAY PROTOCOL

Sample preparation

1. Tissue: According to the ratio of tissue weight (g): isopropano volume (mL) at 1: 5 ~ 10, it is recommended to weigh about 0.1 g tissue and add 1mL isopropano. Homogenize on ice. Centrifuge at 8,000 g for 10 min at 4°C & aspirating the supernatant & place it on ice to be tested.

2. Cell samples: Collect cells into a centrifuge tube. Discard the supernatant after centrifugation. According to the ratio of the cells number (10^4): isopropano volume (mL) at 400 ~ 500: 1, it is recommended to add 1 mL isopropano for every 4~5 × 10^6 cells. Ultrasonically break cells (power 200 W, work 2 seconds, intermittent 1 seconds). Centrifuge at 8,000 g for 10 min at 4°C & aspirating the supernatant & place it on ice to be tested.

3. Serum (plasma) sample: No need to deal with serum (plasma) sample.
4. Plant: According to the ratio of plant weight (g): isopropano volume (mL) at 1: 5 ~ 10, it is recommended to weigh about 0.1 g plant and add 1mL isopropano. Homogenize on ice. Centrifuge at 10,000 g for 10 min at 4°C & aspirating the supernatant & place it on ice to be tested.
Note: For additional measurement, it is recommended to use Abbkine Protein Quantification Kit (BCA Assay) (# KTD3001).

Assay procedure

1. Preheat the microplate reader for more than 30 min, and adjust the wavelength to 420 nm.
2. Preheat the incubator to 65°C.
3. Extraction of TG (The following operations are operated in the EP tube)

	Blank Tube	Standard Tube	Test Tube
distilled water (μL)	40	/	/
Standard (μL)	/	40	/
Serum (μL)	/	/	40
Isopropanol (μL)	125	125	125
Reagent I (μL)	25	25	25

Note: Mix thoroughly after adding isopropanol and then add 25 μL of Reagent I. Shake vigorously for 30 s and let stand. After stratification, transfer 0.15 mL of the upper layer solution to a new EP tube.

4. Triglyceride content determination

	Blank Tube	Standard Tube	Test Tube
upper layer solution (μL)	15	15	15
Isopropanol (μL)	50	50	50
Reagent II (μL)	15	15	15
Mix well and incubate in incubator at 65°C for 3 min			
Reagent III (μL)	50	50	50
Reagent Ⅳ (μL)	50	50	50
Mix well and incubate for more than 15 min at 65°C			

Take out the EP tubes and after cooling down, immediately read optical density at 420 nm. The blank tube is marked as A blank, the standard tube is marked as A standard, and the test tube is marked as A test.
Note: Blank tube and standard tube only need to measure 1-2 times.

DATA ANALYSIS

Calculation of results

Calculation formulae based on 96-well UV plates are as below:

1. Calculation of TG concentration in serum (plasma)

TG (mg/dL) =100×C Standard × (A test-A blank) ÷ (A standard-A blank) = (A test-A blank) ÷(A standard-A blank)

C Standard: 1 mg/mL; 1 dL=100 mL.

2. Calculation of TG concentration in tissues, cells

(1) Calculated by protein concentration

TG (mg/mg prot) = C Standard× V × (A test-A blank) ÷ (A standard-A blank) ÷(Cpr×V) = (A test-A blank) ÷ (A standard-A blank) ÷ Cpr

(2) Calculated by fresh weight of samples

TG (mg/g fresh weight) =C Standard × V × (A test-A blank) ÷ (A standard-A blank) ÷ W = (A test-A blank) ÷ (A standard-A blank) ÷ W

(3) Calculated by cell numbers

TG (mg/10^4 cell) =C Standard × (A test-A blank) ÷ (A standard-A blank) ÷D sample = (A test-A blank) ÷ (A test-A blank) ÷ D sample

Where:

C Standard: 1 mg/mL.

W: fresh weight, g/mL.

V: Isopropanol volume added, 1 mL

Cpr: sample protein concentration, mg/mL

D sample: the density of cells, 10^4 cell /mL.

RELATED PRODUCTS

Product Name	Cat. No.
CheKine™ Free Cholesterol (FC) Colorimetric Assay Kit	KTB2210
CheKine™ Total Cholesterol (TC) Colorimetric Assay Kit	KTB2220